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Feasibility of Extraction and Quantitation of Δ^9 -Tetrahydrocannabinol in Body Fluids by Stir Bar Sorptive Extraction (SBSE) and GC/MS

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ABSTRACT

Proposed revisions to the DHHS Mandatory Guidelines for Federal Workplace Drug Testing Programs [1] (Federal Register, April 13, 2004) include a confirmatory cutoff level of 2.0 ng/mL for THC (parent) in oral fluid. The relatively low cutoff level and small specimen volume (versus the corresponding parameters for urine drug testing) present a challenge to workplace drug testing laboratories. The GC-MS confirmatory procedures for THCA commonly used in certified workplace urine drug testing laboratories lack the sensitivity needed for this application.

We have completed preliminary studies that demonstrate the feasibility of extracting THC from an aqueous matrix using Stir Bar Sorptive Extraction (SBSE) followed by ther-

mal desorption into the injection port of a standard single quadrupole electron impact GC-MS to achieve detection limits less than 1.0 ng/mL. The specimen is diluted with water + methanol and extracted with a stir bar coated with a nonpolar polydimethylsiloxane phase. THC is extracted with high efficiency and minimal matrix interference with no additional sample cleanup needed. THC is introduced onto the GC column by thermal desorption followed by fast GC/MS and selected ion monitoring detection to achieve detection limits in the low ng/mL range.

This technique appears to offer significant advantages of low cost, high extraction efficiency, minimal sample preparation, and compatibility with standard electron impact GC-MS instruments already in common use in workplace drug testing laboratories. With further refinements and optimization for the oral fluid matrix, it may be possible to satisfy the routine confirmatory testing requirements for oral fluid without having to rely on GC-MS-MS or LC-MS-MS techniques to achieve the necessary sensitivity and avoid matrix interference.

INTRODUCTION

Stir Bar Sorptive Extraction has been successfully applied for extraction of drugs of abuse, pharmaceuticals, metabolites, pheromones, bisphenol A and PCB's in a variety of biological fluids (urine [2,3,4,5,6], blood [6], plasma [5], saliva [5], sperm [7], glandular fluid [4]). Proteins, peptides and free triglycerides do not appear to interfere with the extraction of nonpolar target compounds with octanol:water partition coefficients ($\log K_{o/w}$) greater than 2.5. Δ^9 -Tetrahydrocannabinol (THC, Figure 1) exhibits a very high octanol:water partition coefficient ($\log K_{o/w} = 6.97$) and therefore might be expected to partition readily from aqueous solution into the nonpolar PDMS phase during SBSE. Since samples containing analytes in this polarity range can normally be diluted with water or water+alcohol when necessary without negatively affecting the extraction results, SBSE may be a viable extraction approach to confirmatory testing for THC in oral fluid.

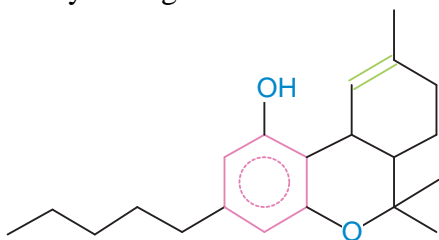


Figure 1. Structure of Δ^9 -tetrahydrocannabinol (THC), the primary active compound in cannabis.

EXPERIMENTAL

Instrumentation. Analyses were performed on a 6890 GC equipped with a 5973 mass selective detector (Agilent Technologies), a PTV inlet (CIS 4) and thermal desorption unit with autosampler (TDS 2 & TDS A or MPS 2 & TDU, GERSTEL).

Analysis conditions.

TDS 2	splitless, 20°C, 60°C/min, 275°C (5 min)
PTV	0.2 min solvent vent solvent vent (50 mL/min), splitless 40°C, 12°C/s, 280°C (3 min)
Column	30m HP5-MS (Agilent) $d_i = 0.25\text{mm}$, $d_f = 0.25\mu\text{m}$
Pneumatics	He, constant flow=1.2mL/min
Oven	60°C; 30°C/min; 175°C; 25°C/min; 300°C (2min)
MSD	scan mode, 31-350 amu

Sample Preparation. Negative oral fluid sample (1.0 g) was weighed into a 10 mL screw cap vial. Nine milliliters of 11% methanol:water containing a known amount of THC were added to the vial. Internal standard ((-)- Δ^9 -THC-D3 in methanol) was added to each sample vial at 5 ng/mL. A Twister stir bar was added and the vial was capped. The sample was extracted for 90 minutes at room temperature. The stir bars were removed, rinsed with bottled water, blotted dry and placed into conditioned thermal desorption tubes for analysis.

RESULTS AND DISCUSSION

Method Optimization. THC standards were prepared in aqueous solutions containing 10-30% (v/v) methanol or acetonitrile. Initial results suggested 10% methanol provided good extraction behavior, so all subsequent extractions were done from 10% methanol. Initial thermal desorption of the Twister stir bars was done at 250°C and a small amount of THC carryover was observed. Desorption temperature was increased to 275°C and interference from THC carryover in the Twister was eliminated.

Extraction Time. THC is a fairly large molecule and therefore might be expected to partition more slowly into the PDMS phase on the Twister stir bar. We tested extraction of THC for 30, 90 and 240 minutes, and found a typical 90 minute extraction achieved nearly quantitative extraction of the THC from a 10 mL sample volume (Figure 2).

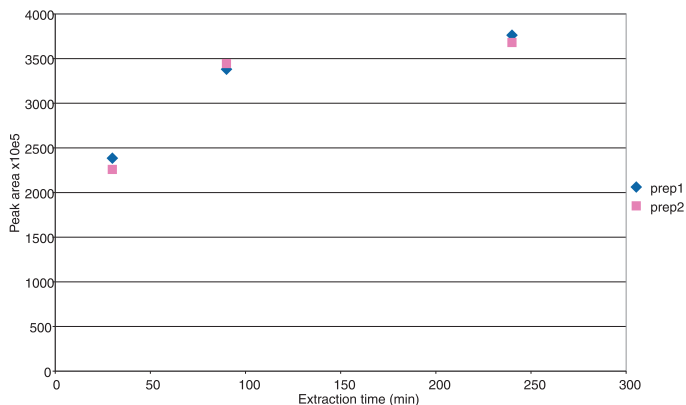


Figure 2. Twister extraction time study for THC spiked into negative oral fluid shows optimal extraction after 90 minutes.

Trapping Temperature. During thermal desorption, the THC is refocused in the cold CIS 4 inlet liner prior to injection onto the GC column. Experiments using trap-

ping temperatures of -120°C , 10°C and 40°C showed THC was trapped well at 40°C , eliminating the need for a cryogen for cooling. This method can therefore be performed on a totally Peltier-cooled GERSTEL TDU/CIS 4+ system.

GC Method. We chose a standard GC column (HP5-MS 30 m x 0.25 mm x 0.25 μm) and full scan detection with the 5973 MSD and obtained good retention and peak shape (Figure 3A). The corresponding extracted 299 ion trace (Figure 3B) showed no interference in this region, which allowed faster temperature ramping of the 6890 GC to achieve THC elution in less than 10 minutes. Preliminary experiments using a GERSTEL MACH fast GC module suggest additional reduction in GC runtime may be possible (data not shown). To achieve the lowest possible detection limits, the method was converted to SIM at 299 using (-)- Δ^9 -THC-D3 as internal standard.

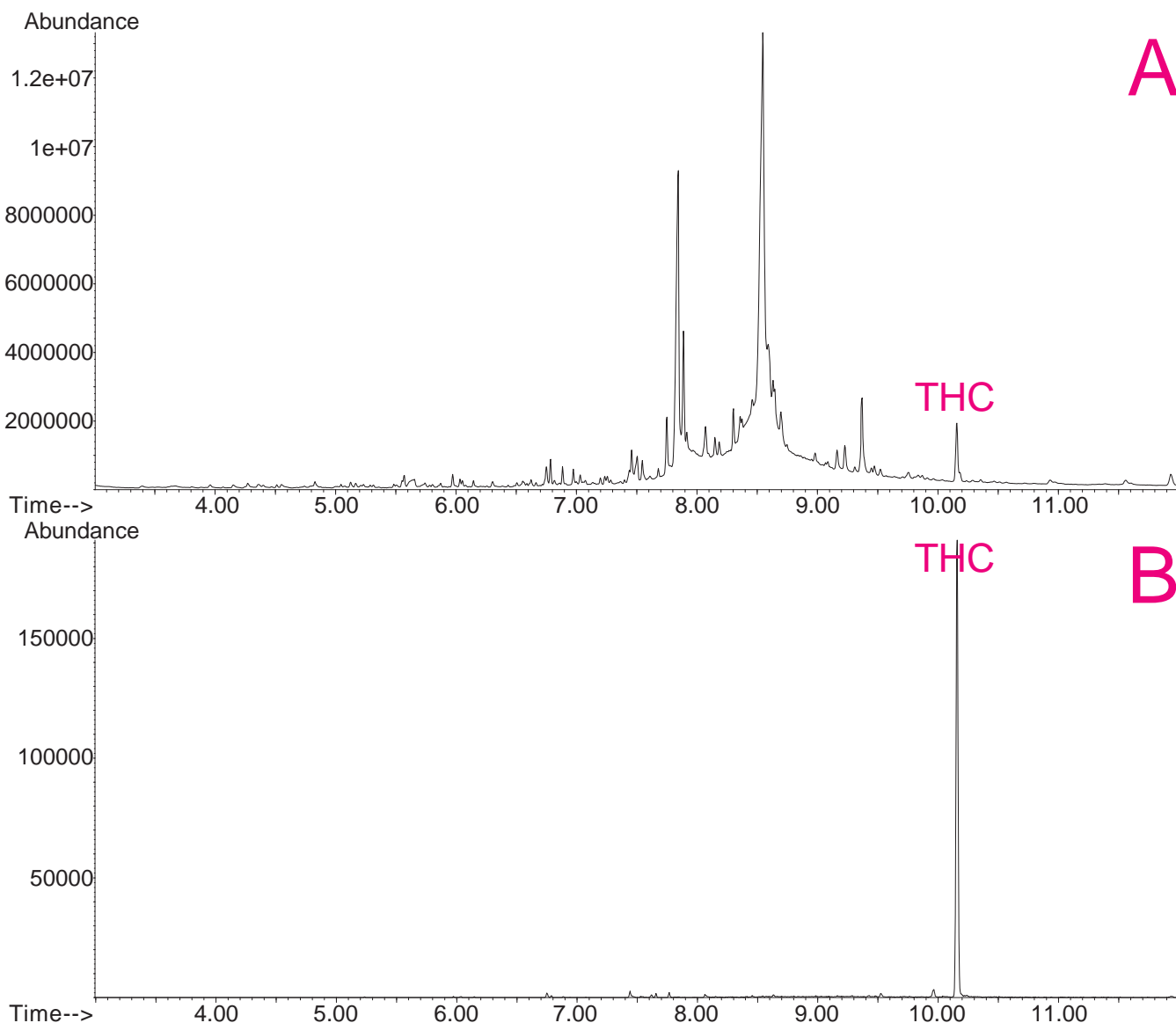


Figure 3. (A) Full scan (31-350 amu) GC/MSD trace for 20 ng/mL spiked THC in oral fluid; (B) Extracted ion trace (299) for 20 ng/mL spiked THC in oral fluid.

Calibration - linear range. Figure 4 shows the 5-point internal standard calibration curve obtained for THC in

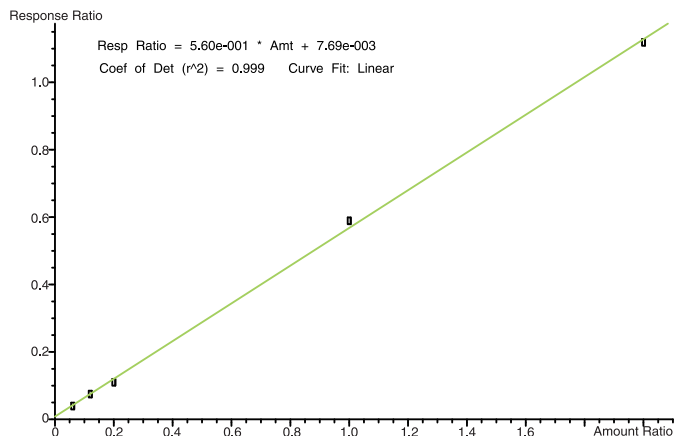


Figure 4. Five point SIM internal standard calibration (0.3 – 10.0 ng/mL THC) in oral fluid.

1.0 g oral fluid. When an internal standard concentration of 5 ng/mL was used, the lowest calibration point (0.3ng/mL in oral fluid) gave a signal approximately 5x larger than the “noise” peak in a negative saliva due to unlabelled THC in the IS. For method validation the IS concentration was reduced to 1 ng/mL.

High Sample Carryover. To assess the potential for carryover, an oral fluid sample was spiked at 40 ng/mL and analyzed. Immediately following the high standard, a blank thermal desorption tube was analyzed under the same desorption conditions. Figure 5A shows an overlay of the 40 ng/mL standard and blank tube; Figure 5B shows an overlay of the blank tube and the lowest calibration level standard (0.3 ng/mL) at a greatly expanded scale. The signal from the carryover blank represents less than 10% of the LOQ standard.

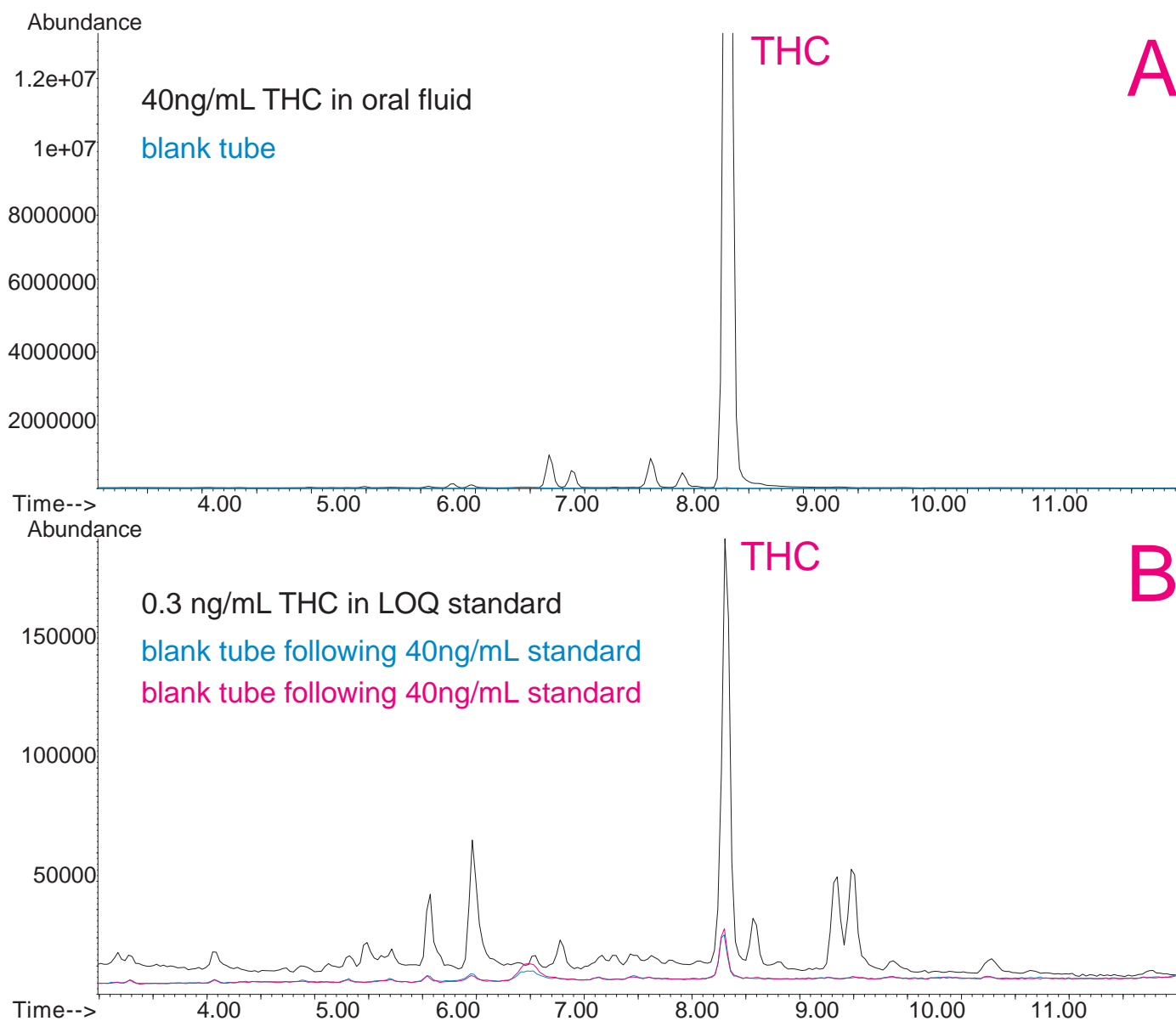


Figure 5. (A) Overlay of 40 ng/mL THC Twister extraction and subsequent blank tube; (B) Overlay of LOQ standard and blank tube following 40 ng/mL high standard shows carryover is negligible.

Twelve negative oral fluid samples from a healthy volunteer were spiked with THC as follows: Five samples spiked at 0.5ng/mL, five samples spiked at 2.0 ng/mL and two samples used as negative controls. The quantitative results obtained for the samples are shown in Table 1.

Table 1. Quantitation of THC in oral fluid.

Sample Number	Spike level (ng/mL)	Result (ng/mL)	%RSD
1	0.5	0.51	
2	0.5	0.51	
3	0.5	0.49	
4	0.5	0.50	
5	0.5	0.49	
	Avg	0.50	2.00
6	2.0	1.99	
7	2.0	2.13	
8	2.0	1.97	
9	2.0	2.00	
10	2.0	2.02	
	Avg	2.02	3.12
11	Negative	ND	NA
12	Negative	ND	NA

ND- Not Detected NA- Not Applicable

Validation and Quantitation. The method was transferred to a second 6890 GC/5975 MSD system with a GERSTEL MPS 2/TDU which has capacity for analysis of up to 196 samples. To meet the requirements for method validation set forth in the NLCP Guidance Document for Laboratories and Inspectors [8], the validation protocol listed in Table 2 was run. The results for the method validation are also shown in Table 2.

Table 2. Method validation with the MPS 2/TDU.

Sample	Concentration	Result	Q Value	Target	Q1	Q2
IS	1.0 ng/mL			302	234	
Calibrator	2.0 ng/mL	2.00 ng/mL		299	231	314
Calibrator	2.0 ng/mL	1.97 ng/mL	97			
Low control	0.8 ng/mL	0.77 ng/mL	96			
High control	2.5 ng/mL	2.46 ng/mL	96			
Unknown	1.2 ng/mL	1.18 ng/mL	97			
Unknown	2.2 ng/mL	2.33 ng/mL	97			
Negative Control 1	0.0 ng/mL	ND				
Negative Control 2	0.0 ng/mL	ND				

Note, based on the initial validation work, the IS concentration was reduced to 1.0 ng/mL to reduce possible interference from unlabelled THC in the IS. The low control (0.8 ng/mL in oral fluid) gave a signal approximately 30x larger than the “noise” peak in a negative oral fluid due to unlabelled THC in the IS.

CONCLUSIONS

Using Stir Bar Sorptive Extraction and analysis by electron impact GC-MS with selected ion monitoring, it is possible to reliably extract and quantify THC in oral fluid at concentrations comfortably below the cutoff levels that have been proposed for confirmatory testing of alternative specimens in federal workplace drug testing programs. With this approach, the same confirmatory testing parameters (chromatography, resolution, three selected ions with acceptable ion abundance ratios, quality control requirements, LOD/LOQ, etc.) that have been applied successfully for confirmatory testing of drugs in urine by the National Laboratory Certification Program for almost 20 years could be extended to the more demanding analysis of THC in oral fluid.

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